Research Journal of Agriculture and Biological Sciences, 8(2): 224-230, 2012
ISSN 1816-1561
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Biological Control of Date Palm Root Rots Disease Using Egyptian Isolates of Streptomycetes

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ABSTRACT

Date palm trees are exposed to infection with different diseases caused by many soil-borne pathogenic fungi that may cause considerable losses in the offshoots and trees. This study was carried out on five cvs. of date palm during two years in Aswan and New Valley governorates. Isolation and identification of associated fungi showed that the most frequent fungi were *Fusarium oxysporum*, *F. moniliforme* and *Thielaviopsis paradoxa* while the least frequent was *Phytophthora* sp. The cultivars were differed susceptible to infection by the isolated pathogenic fungi. Barhey cultivar was the most susceptible to infection, followed by Gondaila, Siwey and Partmoda cultivars, while Sakkoty cultivar was the least susceptible. Seven streptomycetes were isolated from the rhizosphere soil of healthy date palm cultivated in Aswan governorate. Streptomycetes were namated *viz*. *Streptomyces bobilii* (isolate A1, A5 and A7), *Streptomyces grisiobrunneus* (isolate A2 and A3), *Streptomyces albolongus* (isolate A4 and A6). Three isolates A1, A6 and A3 of them gave highest results *in vitro* and in greenhouse experiments against soil-borne pathogenic fungi compared with negative and positive control. The obtained results showed that Streptomyces had the highest efficacy on root rots of date palm. This is the first study done to use actinomycetes against soil-borne pathogenic fungi that caused root rots of date palm.

Key words: date palm, soil-borne pathogenic fungi, Actinomycetes, biological control, root rots

Introduction

Date palm (*Phoenix dactylifera* L.) roots are liable to attack by many pathogenic soil borne fungi, that causing serious diseases. Several fungi were recorded as causal pathogens of root rot on date palm (Mansoori and Kord, 2006; El Deeb *et al*., 2007; Samir *et al*., 2009; Arafat, 2011). Some genera such as *Streptomyces* and *Actinoadura* are widely distributed, which can be isolated from different temperate soil habitats (Williams *et al*., 1989). The majority of the Streptomyces belonging to actinomycetes were active against *Fusarium solani* and *Botrytis cinerea* (Tulemisova and Chormonova**,** 1989**)**. Actinomycetes are peerless sources of bio-active metabolites including antibiotics, plant growth factors, and other substances are the effective against phytopathogenic fungi (Bressan, 2003 and Shahidi *et al*., 2004). Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance (Ramanathan *et al*., 2002). Hydrolases such as chitinase contribute to degradation of fungal cell walls (Korsten *et al*., 1994). Chitin is the second most abundant polysaccharide in nature and a major component of fungal walls, insect exoskeletons and crustacean shells. Chitinase secreted by a BCA is likely to be effective against pathogenic fungi, the cell walls of which are mainly chitin. This study aimed to evaluate the ability of the Egyptian isolated Streptomycetes to inhibit *in vitro* soil-borne pathogenic fungi and the efficacy of promising antagonistic isolates to reduce in greenhouse the incidence of root rots induced by pathogenic fungi on date palm.

Materials and Methods

Isolation and identification of microorganisms associated with root rotted:

Samples of root rots of date of the dry cultivars *i.e*., Gondalia, Partomoda and Sakkoty were collected from Aswan governorate, while semi-dry cultivars *i.e*., Barhey and Siwey were collected from New Valley governorate. Disease incidence was carried out according to Cooke *et al*. (2006). Samples were taken at least 15-20 cm apart then were washed carefully and surface sterilized with 1% sodium hypochlorite solution for 2 mins. The species of sterilized root were transferred on potato dextrose agar (PDA) plates and incubated at

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25±2°C. Emerged fungi were isolated and purified using the single spore technique and/or the hyphal tip method according to Wang and Wen (1997). Stock cultures were maintained on PDA slants and kept in a refrigerator at 5°C. for further studies. The fungal colonies growing in the culture plates were identified according to their morphological characteristics according to Barnett and Hunter (1999) and John and Summerell (2006). The frequency of the isolated fungi from the root rotted samples was separately calculated according to the following formula: % Fungal frequency= No. of isolates of each fungs/$\sum$ of all isolates X 100.

Isolation and identification of Streptomycetes:

Rhizosphere soil samples were collected under date palm trees healthy growing in Aswan governorate. The plate count method was used for the isolation of Streptomycetes according to the method of Seong *et al.,* (2001). These Plates were incubated for 2-7 days at 25^{o}C and checked for the growth of the desired Streptomycetes colonies after incubation. The isolated Streptomycetes were identified by National organization of Medication Inspection and Research, Ministry of Health according to Shirling and Gottlieb (1966).

Pathogenicity test:

Pathogenicity test of the isolated fungi, *viz. F. oxysproum* Schlecht, *F. moniliforme* Sheldon, *T. paradoxa* Peyr. and *Phytophthora* sp. which were isolated from diseased roots was carried out in the greenhouse of Fruit and Woody Trees Diseases Research Department-Agric. Res. Center, Giza-Egypt. The seeds of date palm were surface- disinfested for 10 min in a sodium hypochlorite solution NaOCl (1.5% available chlorine), then soaked under tap water for 24 h, and dried. The sterilized seeds were sown in black plastic bags (15 cm) filled with a sterilized mixture soil of equal portions (v/v) of sand and clay. The seedlings were allowed to grow for 6 months or to the
2-3 leaves-stages. Five bags (each contained one date palm) representing each of the tested cultivars, *i.e*., Barhey, Gondaila, Partomoda, Sakkoty and Siwey were used as replicates for each tested fungus.

In vitro antagonistic bioassay:

The Streptomycetes isolates were evaluated for their activity against soil-borne pathogenic fungi, Discs of Actinomycetes (5 mm) were remove from the edge colonies of active cultures and placed on one side of a Petri dishes containing Potato Dextrose Agar (PDA) medium. Similar dishes of each pathogenic fungus isolates grown in the same manner were placed on the opposite side of Petri plates and made three replicates. Cultures were observed daily and recorded for antagonism of Streptomycetes isolates against pathogenic fungi. Experiments were repeated twice for the most antagonistic of Streptomycetes isolates to evaluate reproducibility.

Greenhouse experiments:

Evaluation the efficacy of approaches of Streptomycetes against root rot incidence of date palm cultivars was carried out in pot experiment in the open greenhouse of Fruit and Woody Trees Diseases Research Department-Agric. Res. Center, Giza-Egypt. The evaluated treatments were applied soil drench. Seedlings of date palm cultivars *i.e.,* Gondalia, Siwey and Barhey at six months age were inoculated with tested fungi *viz*. *F. moniliforme* and *T. paradoxa* spore suspension at ($1X10^6$ spores/ml) for each fungus. After one week of incubation, spore suspension at ($1X10^6$ spores/ml) of Streptomycetes isolate was inoculated into each pot, except for (C^- as negative control) treated with water and (C^+ as positive control) treated with fungicide (Topsin M 70%{Thiophanate-methyl} at 3g/l as soil drench treatment). Incubation was continued for further 30 days. Three pots were used for each treatment and repeated three times. Disease incidence percentage of plants was visually recorded at 30 days after inoculation.

Statistical Analysis:

All experiments were set up in a complete randomized design. The results of experiments were analyzed by CoStat software ver. 6.4-CoHort. The mean of treatments were compared by LSD at $P < 0.05$ level of probability according to (Gary, 2010).

Results:

Isolation and identification of fungi associated with root rotted:

The disease survey of root rots on date palm was carried out during continuous two years 2011-2012 in Aswan and New Valley governorates. Disease incidence was recorded in roots and rhizosphere soil samples which collected for isolation and identification of fungi. Data in Table (1) showed that *F. oxysporum* was the most frequency fungus from Aswan governorate ranged (40%), followed by *F. moniliforme* ranged (30%) and *T. paradoxa* ranged (25%). While *Phytophthora* sp. was ranged (5%). On the other hand, *T. paradoxa* was the most frequency in New Valley governorate (35%), followed by *F. oxysporum* (30%) and *F. moniliforme* (25%). While the least fungus was *Phytophthora sp*. (10%) in New Valley. Disease incidence was recorded (40% and
25%) in Aswan and New Valley governorates, respectively.

Table 1: Frequency of fungi isolated from date palm root rots

Governorate	Associated fungi %				Disease incidence%
	F. oxysporum	*F. moniliforme*	*T. paradoxa*	*Phytophthora* sp.	
Aswan	40	30	25	5	40
New Valley	30	25	35	10	25

Isolation and identification of Streptomycetes:

Screening of Streptomycetes isolated from rhizosphere soil of date palm indicated that, seven strains of actinomycetes were identified *i.e.*, *Streptomyces bobilii* (isolate A1, A5 and A7), *Streptomyces grisiobrunneus* (isolate A2 and A3), *Streptomyces albolongus* (isolate A4 and A6). The percentage of frequency of Streptomycetes were *Streptomyces bobilii* (A1, 35%), *Streptomyces grisiobrunneus* (A2, 5%), *Streptomyces grisiobrunneus* (A3, 15%), *Streptomyces albolongus* (A4, 20%), *Streptomyces bobilii* (A5, 5%), *Streptomyces albolongus* (A6, 10%) and *Streptomyces bobilii* (A7, 10%). (Fig. 1).

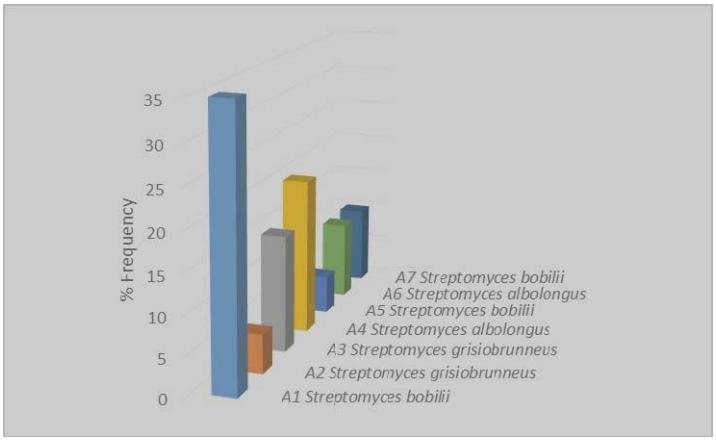

Fig. 1: Frequency of actinomycetes isolated from rhizosphere soil of date palm

Pathogenicity test:

Data in Table (2) showed that, the most virulent fungus was *T. paradoxa* (D.I. 42%) followed by *Phytophthora* sp. and *F. moniliforme* (D.I. 32.9 and 30.4%, respectively). On the other hand, *F. oxysporum* was the latest one (D.I. 25.6%), in all cultivars studied. Barhey cultivar was the most susceptible to infection by all fungi studied in two trials test (D.I. 51 and 51%, respectively), followed by Gondaila (D.I. 38.7 and 41%), Siwey (D.I. 33 and 36.7%) and Partomoda (D.I. 26.3 and 37%), respectively. On the contrary, Sakkoty (D.I. 7.3 and 5.3%) was the latest cultivar susceptible to infected with all fungi under studied.

In vitro antagonistic bioassay:

All Streptomycetes strains that were tested for antagonists of soil-borne fungi in lab. (Table 3) affective expect two strains, but with different response as inhibition zone. Streptomycetes strain (A4) showed strong antagonism with against all fungi tested with (1.29 cm of inhibition zone), followed by (A1) ranged (Iz 1.09 cm). (A6 and A3) moderate antagonism with (0.83 and 0.76 cm of inhibition zone, respectively). While, (A7) was the weak effective ranged (Iz 0.37 cm). On the other hand, *Phytophthora* sp. was the most impressed by all actinomycetes tested (Iz 1.07 cm) followed by *F. moniliforme* and *F. oxysporum* ranged (Iz 0.89 and 0.61 cm, respectively). While *T. paradoxa* was the weak affected by all actinomycetes tested (Iz 0.32 cm).

Table 2: Pathogenicty test of the isolates fungi from the root rots on the date palm cultivars seedlings.

Cultivars / Fungi	Sakkoty		Partomoda		Gondaila		Siwey		Barhey		M.
	% Disease incidence										
	R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2	
Phytophthora sp.	0.0	0.0	0.0	0.0	44.0	60.0	24.0	36.0	60.0	60.0	28.4
T. paradoxa	0.0	0.0	61.3	65.3	62.7	61.3	40.0	42.7	41.3	45.3	42.0
F. moniliforme	0.0	0.0	44.0	37.3	25.3	22.7	45.3	45.3	41.3	42.7	30.4
F. oxysporum	29.3	21.3	0.0	0.0	22.7	20.0	22.7	22.7	61.3	56.0	25.6
Mean	7.3	5.3	26.3	25.7	38.7	41.0	33.0	36.7	51.0	51.0	31.6

L.S.D. at 0.05=
Fungi (F) = 1.86 Cultivars (C) = 2.08 R = ns.
F X C = 4.15 F X R = 2.63 C X R=ns.
F X C X R = 5.87

Greenhouse experiments:

The disease incidence percentage of root rots are presented in Table (4) presented data showed that; the applied with Streptomycetes were efficacy for reducing root rots of date palm seedlings. Data the percentage of disease incidence on seedlings treatment by *Streptomyces bobilli* (A1) was the highly reduce root rots (D.I. 30.45%) caused by two fungi tested on date pam cultivars tested, followed by *Streptomyces grisiobrunneus* (D.I. 41.12%), while *Streptomyces albolongus* was the least one to reduce the percentage of disease incidence (D.I. 58.89%) compared with control negative (D.I. 98.89%) and control positive (D.I. 13.89%). On the other hand, Ganoderma and Siwey cultivars were the most affective by Streptomycetes treatment, while Barhey was the least cultivar affective by actinomycetes treatment.

Discussion:

Date palm trees under the Egyptian conditions are subjected to infection with different diseases caused by many soil-borne pathogenic fungi causing considerable root rots in the orchards. Soil-borne pathogenic fungi *viz*. *F.oxysporum*, *F*. *moniliforme*, *T. paradoxa* and *Phytophthora* sp. caused root rots of date palm trees in governorates under study, Aswan and New Valley in Egypt. (Abbas *et al*., 1989; El-Deeb, 1994). *F*. *oxysporum*, *F*. *moniliforme* and *T. paradoxa* were the most frequently in all locations under studied in Aswan and New Valley governorates, while the least one was *Phytophthora* sp. The occurrence and frequency of the isolated fungi were differed from one location to another; these differences are probably due to the environmental conditions such as moisture, temperature and soil type, dissemination factors of fungi in different locations, cultivars of date palm and agricultural practices. These results are in harmony with those obtained by Fawcett and Klotz (1932). Variations were recorded on the disease incidence percentage in the greenhouse between different cultivars. These results are in agreement with those obtained by El-Deep *et al*. (2007). It may be also due to one or more of the following factors; pathogen frequency, climatic conditions that differ considerably between locations, cultivars sensitivity, dissemination factors available in the locality and it may be also due to the cultural practices (Turner, 1981; Arafat, 2011). *Fusarium* spp. were the most frequently isolated fungi from two governorates studied. On the other hand, *T. paradoxa* was consistently isolated from rotted roots of date palm; these results are in agreement with those obtained by Samir *et al*. (2009) and Arafat (2011). On the contrary, Djerbi (1991) reported that *T. paradoxa* was not isolated from the roots of naturally infested trees. This may be due to the presence of various biotypes of the fungus in different regions (Al-Rokibah *et al*., 1998). The pathogenic potentialities of the isolated fungi were estimated with five date palm cultivars in the greenhouse, all seedlings of date palm cultivars were infected with all soil-borne pathogenic fungi, but with various degrees of susceptibility. The most virulent fungus was *T. paradoxa*, followed by *Phytophthora* sp. and *Fusarium moniliforme* while *F*. *oxysporum* was weak virulent to date palm. Barhey cultivar was the most susceptible to root rot followed by Gondaila, Siwey and Partomoda cultivars, respectively. While, Sakkoty cultivar was the less susceptible. Virulent of the isolated fungi were estimating in the greenhouse trials with susceptible cultivars under studied. All isolated fungi were found pathogenic with all cultivars under studied, but with different degrees of virulent and susceptible. The most virulent fungus was *T. paradoxa*; this result is in agreement with (Arafat, 2011) who mentioned that the most virulent fungi were *Fusarium* spp. Followed by *T. paradoxa* on different cultivars *viz*. Zaghloul, Sammany and Hayany. A high correlation between actinomycetes and antifungal properties has been reported (Jung *et al*., 2003 and Hoster *et al*., 2005). In this study, we isolated seven actinomycete strains from rhizoshere soils using dilution method. Five strains exhibited strong inhibition activity as determined by inhibition zone on PDA agar. All selected strains were then screened for antagonistic properties against root rots fungi *viz*. *T. paradoxa* and *F. moniliforme in vitro*. Five of actinomycetes isolates were active against all of the soil-borne pathogenic fungi. All the active isolates were identified as Streptomyces sp. Lemanceau *et al*. (1995) and Wiehe *et al*. (1996) indicated that differences in the quantitative and qualitative composition of root excretions provide different impact on the rhizosphere microbiota and attract more or less

bacterial antagonists responsible for natural soil suppression. Plant root exudates stimulate growth of rhizosphere Actinomycetes that are strongly antagonistic to fungal pathogens, while the actinomycetes utilize root exudates for growth and synthesis of antimicrobial substances (Crawford *et al*. 1993; Yuan and Crawford 1995). The present study showed that *Streptomyces bobilli* (A1), *Streptomyces grisiobrunneus* and *Streptomyces albolongus* were significant antagonism of fungi *in vitro* assay but not quite active in greenhouse. It is possible that the condition in pot experiments may not promote the growth of these strains or these strains probably could not establish antagonistic mechanisms in the soil. The results indicated that antibiosis *in vitro* was not related to the disease reduction *in vivo*. On the other hand, the pattern of antagonism, by the most active actinomycete strains, indicated the production of water-soluble antifungal metabolites, since large zones of inhibition were evident on the PDA plates. However, it is possible that the mechanism of antagonism may have involved neither in the production and excretion of chitinolytic enzymes to inhibit the growth of pathogenic fungi nor the production of secondary metabolites. Of one thousand different antibiotics known today, more than 70 % are produced by *Actinomycetes* (Kim & Garson, 2005). Hoster *et al.* (2005) reported that the chitinase of *Streptomyces griseus* possessed antifungal activity against the following fungi; *Aspergillus*, *Botrytis cinerea*, *Fusarium culmorum, Guignardia bidwellii,* and *Sclerotia sclerotiorum.* Liu *et al.* (1996) concluded that the ability to suppress the growth of pathogen was not related to the reduction of tomato scab disease in field study by *Streptomyces* spp. Anees *et al.* (2010) also suggested that different antagonistic mechanisms were evident for different strains and the ability to produce water-soluble inhibitors or coil around the hyphae of the pathogen *in vitro* was not related to the disease reduction *in vivo*. In the present study, the most efficient strain overall was *Streptomyces bobilli* (A1) which was able to reduce the growth of the pathogen and suppress the disease both *in vitro* and *in vivo*. As a whole, our data suggest that *Streptomyces bobilli* (A1) might be an effective antagonist applicable to the control of the root rots of date palm caused by soil-borne pathogenic fungi. However, investigation that is more detailed is required to demonstrate the potential of these organisms for the biocontrol of pathogenic fungi and in plant growth promotion that may be useful as biological control agents (BCA) in the future.

Table 3: Inhibition of Streptomyces Effect of Actinomycetes strains on pathogenic fungi *in vitro*.

A. Fungi	A1	A2	A3	A4	A5	A6	A7	Control	Mean
	Inhibition zone (cm)								
F. moniliforme	1.53	0.00	1.17	1.13	0.00	1.20	0.33	0.00	0.89
F. oxysporum	0.93	0.00	0.53	1.20	0.00	0.27	0.70	0.00	0.61
T. paradoxa	0.20	0.00	0.13	1.40	0.00	0.10	0.07	0.00	0.32
Phytophthora sp.	1.70	0.00	1.20	1.43	0.00	1.13	0.37	0.00	0.97
Mean	1.09	0.00	0.76	1.29	0.00	0.68	0.37	0.00	0.70

L.S.D. at 0.05=
Fungi (F) = 0.09 Streptomycetes (A) = 0.12 F X A= 0.25

Table 4: Effect Disease inicidence percentage of root rots date palm caused by soil-borne fungi treated with Streptomycetes

Fungi	% Disease incidence										Mean
	Thielaviopsis paradoxa					*Fusarium moniliforme*					
	Treatments					Treatments					
Cultivars	A1	A3	A4	C^-	C^+	A1	A3	A4	C^-	C^+	
Ganoderma	16.67	40.00	26.67	66.67	7.67	11.67	26.67	33.33	53.33	11.33	29.40
Siwey	53.33	33.33	13.33	66.67	9.33	14.33	40.00	33.33	73.33	12.67	34.97
Barhey	53.33	40.00	73.33	53.33	7.33	33.33	66.67	60.00	93.33	10.67	49.31
Mean	41.11	37.78	37.78	62.22	8.11	19.78	44.45	42.22	73.33	11.56	37.83

L.S.D. at 0.05=
Treatments (T) = 7.91 Fungi (F) = ns. Cultivars (C) = 6.13
T X F = 33.55 T X C = 27.40 F X C = ns.
T X F X C = 19.37

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